

**In the specification**

Please amend paragraph 1, on page 1, as follows:

This application is a continuation of U.S. Application No. 09/796,988, filed February 28, 2001, allowed, which claims the benefit of Provisional Application Ser. No. 60/186,046, filed March 1, 2000; and is a continuation-in-part of U.S. Application No. 09/724,959, filed November 28, 2000; and is a continuation-in-part of U.S. Application No. 09/640,953, filed August 16, 2000; which is a continuation of U.S. Application Ser. No. 09/054,832, filed April 3, 1998; and is a continuation-in-part of U.S. Application Ser. No. 09/431,[[4]]385, filed November 1, 1999; which is a continuation of U.S. Application Ser. No. 09/054,830, filed April 3, 1998, now U.S. Patent No. 6,127,121; the disclosures of each being incorporated herein by reference.

Please rewrite lines 22-29, on page 3 as follows:

Figure 3 A-B is a chart which illustrates one advantage achieved through the use of PPPA and PPG in MGB-modified oligonucleotide probes. As seen in the figure, the modified bases allow shortening of the probe that shows increased mismatch discrimination in real-time PCR. Ã is PPPA and Ĝ is PPG. Panel A shows a first design of a 18-mer fluorescein-ODN-Red 13 quencher-MGB real-time PCR probe, with poor discrimination of an A/T mismatch. Panel B shows a re-design probe against the opposite strand, putting the mismatch under the MGB and substituting PPPA for A and PPG for G as indicated, which allows shortening of the probe to a 15-mer. MGB-modified FAM probe = SEQ ID NO:1; MGB-modified FAM probe complement = SEQ ID NO:2; PPPA and PPG containing MGB-modified FAM probe complement = SEQ ID NO:3; PPPA and PPG containing MGB-modified FAM probe = SEQ ID NO:4.

Figure 4 A-B illustrates an Invader™ assay in which the modified oligonucleotides of the invention can be used. Panel A shows the enzyme cleaves the overhanging "flap", which serves as an invader probe in the detection cassette probe where cleavage releases a fluorescence signal. The first cleavage takes places only when the single base mismatch in the invader is a perfect match. Panel B shows that no reaction takes place with a mismatch target.

Figure 5 A-C illustrates a comparison of Invader™ probe performance with different numbers of PPGs. (SEQ ID NOS: 5-7). Panel A shows six Gs substituted with PPG; Panel B shows one G substituted with PPG and Panel C shows no G substituted with PPG.

Please replace the paragraph (Table 1) on page 40, line 6, with the following:

**Table 1**  
**Sequences of Oligonucleotide Probes and Complementary Targets**

<u>Complementary Targets</u>	<u>SEQ ID</u> <u>NO:</u>	<u>Py/Pu-rich probes</u>	<u>base</u> <u>pairs</u>
1* TCGGCGGCGT	<u>8</u>	1*.MGB-Q-CGCCGCCG	8 G/C
2* ACAGCGGCGT	<u>9</u>	2* MGB-Q-CGCCGCTG	7 G/C, 1 A/T
3* ACAGCGACGT	<u>10</u>	3*.MGB-Q-CGTCGCTG	6 G/C, 2 A/T
4* TCAGTGACGA	<u>11</u>	4*.MGB-Q-CGTCACTG	5 G/C, 3 A/T
5* TCAGTGACAA	<u>12</u>	5*.MGB-Q-TGTCACTG	4 G/C, 4 A/T
6* TCAATGACAG	<u>13</u>	6*.MGB-Q-TGTCATTG	3 G/C, 5 A/T
7* ACAATGATAA	<u>14</u>	7*.MGB-Q-TATCATTG	2 G/C, 6 A/T
8* CCAATAATAA	<u>15</u>	8*.MGB-Q-TATTATTG	1 G/C, 7 A/T
9* GTAATAATAA	<u>16</u>	9*.MGB-Q-TATTATTA	8 A/T

Please replace the paragraph (Table 3a and 3b) on page 83, line 14, with the following:

**Table 3a and 3b**  
**Probe and target sequences**

<b>A. Probe Sequences – Mismatch Underlined</b>			
<b>Number</b>	<b>Mismatch</b>	<b>Probe Sequence</b>	<b><u>SEQ ID</u> <u>NO:</u></b>
1	Complement	AAAGTTATGTCTACTTACAGAAA	<u>17</u>
2	A/C	AAAG <u>C</u> TATGTCTACTTACAGAAA	<u>18</u>
3	A/C	AAAGT <u>C</u> ATGTCTACTTACAGAAA	<u>19</u>
4	T/G	AAAGTTG <u>T</u> GTCTACTTACAGAAA	<u>20</u>
5	A/C	AAAGTTACG <u>T</u> CTACTTACAGAAA	<u>21</u>
6	C/A	AAAGTTATAT <u>C</u> TACTTACAGAAA	<u>22</u>
7	A/C	AAAGTTATGCCTACTTACAGAAA	<u>23</u>
8	G/T	AAAGTTATGTT <u>A</u> CTTACAGAAA	<u>24</u>

9	A/C	AAAGTTATGTCC <u>ACT</u> TACAGAAA	<u>25</u>
10	T/G	AAAGTTATGTCT <u>GCT</u> TACAGAAA	<u>26</u>
11	G/T	AAAGTTATGTCTATTTACAGAAA	<u>27</u>
12	A/C	AAAGTTATGTCTAC <u>CT</u> TACAGAAA	<u>28</u>
13	A/C	AAAGTTATGTCTACT <u>CAC</u> AGAAA	<u>29</u>
14	T/G	AAAGTTATGTCTACTT <u>G</u> CAGAAA	<u>30</u>
<b>B. Target Sequences – A'=PPPA</b>			
1		GTAAGTAGACATAAC	<u>31</u>
2		GTA'A'GTA'GA'CA'TA'A'C	<u>32</u>
3		GTAAGTAGACATAAC-MGB	<u>33</u>
4		GTA'A'GTA'GA'CA'TA'A'C-MGB	<u>34</u>

Please replace paragraph (Table 4) on page 85, line 1, with the following:

**Table 4**

**Comparison of thermodynamic discrimination of mismatched base pairs formed by HOPPPA or HOPU vs PPPA and PU in the 8-mer duplexes (+MGB).**

	Sequence of Duplex	<u>SEQ ID NO:</u>	PPPA/ PU	HOPPPA/ HOPU		Sequence of Duplex	<u>SEQ ID NO:</u>	PPPA/ PU	HOPPPA/ HOPU
			$\Delta\Delta G$ cal/mol	$\Delta\Delta G$ cal/mol				$\Delta\Delta G$ cal/mol	$\Delta\Delta G$ cal/mol
Match	CGUCACUG-MGB AGCTGTGACT	<u>35</u>			Match	UAUUAUUG-MGB AATAATAACC	<u>45</u>		
1	CGUCACUG-MGB AGCTGTGACT	<u>36</u>	4250	4350	10	UAUUAUUG-MGB AATTATAACC	<u>46</u>	4400	5000
2	CGUCACUG-MGB AGCGGTGACT	<u>37</u>	3450	3540	11	UAUUAUUG-MGB AATGATAACC	<u>47</u>	3740	3760
3	CGUCACUG-MGB AGCCGTGACT	<u>38</u>	4860	4530	12	UAUUAUUG-MGB AATCATAACC	<u>48</u>	6630	6840
4	CGUCACUG-MGB AGCAGAGACT	<u>39</u>	4870	4850	13	UAUUAUUG-MGB AATAAAAACC	<u>49</u>	5090	5730
5	CGUCACUG-MGB AGCAGGGACT	<u>40</u>	4190	4360	14	UAUUAUUG-MGB AATAAGAACC	<u>50</u>	5920	6520
6	CGUCACUG-MGB AGCAGCGACT	<u>41</u>	3930	3940	15	UAUUAUUG-MGB AATAACAACC	<u>51</u>	4120	4530
7	CGUCACUG-MGB AGCAATGACT	<u>42</u>	2600	2300					
8	CGUCACUG-MGB AGCATTGACT	<u>43</u>	4360	4210					
9	CGUCACUG-MGB AGCACTGACT	<u>44</u>	4420	4610					

$\Delta\Delta G$  was calculated at 37°C.

Please replace the paragraph (Table 5, heading) on page 87, at line 6 as follows:

Table 5

Comparison of  $^{32}\text{P}$ -incorporation in primer extension product by polyacrylamide gel electrophoresis using AAC CAC TCT GTC CTA (SEQ ID NO:52) template

Please replace the paragraph (Table 6) beginning on page 88, line 1, as follows:

Table 6

Comparison of experimental  $T_m$ s with that of predicted  $T_m$ s using the nearest-neighbor thermodynamic parameters for PPG containing oligonucleotides and PPG containing oligonucleotides attached to a MGB

5'-Probe Sequence -3'	SEQ ID NO:	ODN Duplex Stability °C			MGB-ODN Duplex Stability °C		
		$T_{m_{exp}}$	$T_{m_{calc}}$	Err	$T_{m_{exp}}$	$T_{m_{calc}}$	Err
CTGTAAGTAGATATAAC	<u>53</u>	51.84	53.23	1.39	65.88	66.69	0.81
GGCAAGATATATAG	<u>54</u>	50.21	49.81	-0.40	66.37	65.56	-0.81
GTGACGCAGATTCC	<u>55</u>	61.27	61.06	-0.21	76.97	75.19	-1.78
GTAAGTAGACATAAC	<u>56</u>	52.12	51.78	-0.34	64.64	63.31	-1.33
CAGGGAGCTTTGGA	<u>57</u>	59.9	60.22	0.32	74.39	71.47	-2.92
CACTCGTGAAGCTG	<u>58</u>	60.85	59.49	-1.36	74.04	72.26	-1.78
GTAAGTAGGCATAAC	<u>59</u>	55.74	55.47	-0.27	66.91	66.00	-0.91
CCGGATGTAGGATC	<u>60</u>	57.52	59.05	1.53	69.3	70.03	0.73
GATTACCTGGATTT	<u>61</u>	50.64	50.32	-0.32	62.29	62.33	0.04
CCGTCAATGGTCAC	<u>62</u>	58.66	60.01	1.35	70.13	69.91	-0.22
CAGCACGTAGCC	<u>63</u>	57.31	58.07	0.76	69.29	67.60	-1.69
CGGCTACGTGCTGG	<u>64</u>	65.19	66.01	0.82	76.12	74.79	-1.33
CGGCTACATGCTGG	<u>65</u>	61.14	61.95	0.81	71.56	72.99	1.43
CTAAATCTGCCG	<u>66</u>	50.4	48.09	-2.31	62.08	60.19	-1.89
TCTGGATGATGGGCA	<u>67</u>	61.74	61.95	0.21	71.65	72.13	0.48
GTTTCATGGGTGTAAT	<u>68</u>	57.51	57.77	0.26	66.94	68.79	1.85
CGGAGGTAGGATCA	<u>69</u>	59.24	59.46	0.22	69.46	70.93	1.47
CCACCCGCCTCAG	<u>70</u>	60.73	61.14	0.41	71.43	70.74	-0.69
CACAGGAGTGCTTGG	<u>71</u>	63.07	64.40	1.33	72.28	72.92	0.64
CGGACCACTGCGTG	<u>72</u>	68.1	67.58	-0.52	77.92	76.80	-1.12
TCGGACCACTGCGT	<u>73</u>	65.04	66.00	0.96	74.94	75.62	0.68
AACGGGGTACGATA	<u>74</u>	57.93	57.11	-0.82	67.79	67.08	-0.71
CAGTTGAGATTCTAAGAC	<u>75</u>	60.06	60.15	0.09	67.15	67.43	0.28
AGGGGCGTCTTG	<u>76</u>	60.78	58.57	-2.21	71.62	72.76	1.14
GTAAGTAGGCATAGC	<u>77</u>	58.34	58.95	0.61	65.95	66.99	1.04

TGCCCAGCCCCAG	<u>78</u>	<b>63.13</b>	63.40	0.27	<b>71.28</b>	71.32	0.04
CCAACACTCGTGAA	<u>79</u>	<b>54.87</b>	56.14	1.27	<b>62.07</b>	63.54	1.47
GTAAGTAGACACAGC	<u>80</u>	<b>59.48</b>	58.41	-1.07	<b>65.79</b>	66.27	0.48
TCGGACCAGTGC	<u>81</u>	<b>58.02</b>	58.55	0.53	<b>65.99</b>	66.35	0.36
CGATCACGCTGGC	<u>82</u>	<b>62.12</b>	62.75	0.63	<b>69.18</b>	71.81	2.63
GTCCTGGGGGTGG	<u>83</u>	<b>65.19</b>	64.54	-0.65	<b>72.78</b>	72.53	-0.25
GTAAGTAGGTGTGAC	<u>84</u>	<b>60.7</b>	59.70	-1.00	<b>66.92</b>	67.00	0.08
GGTTGTACGGGTTACG	<u>85</u>	<b>68.38</b>	68.81	0.43	<b>74.16</b>	75.38	1.22
GGACCAGTGCGTGA	<u>86</u>	<b>66.84</b>	65.46	-1.38	<b>73.38</b>	71.53	-1.85
GTAAGTAGACGCAGC	<u>87</u>	<b>62.91</b>	62.44	-0.47	<b>68</b>	67.82	-0.18
GTAAGTAGGCGCAGC	<u>88</u>	<b>65.52</b>	65.91	0.39	<b>69.8</b>	70.34	0.54
GTAAGTAGGCGCGGC	<u>89</u>	<b>68.71</b>	68.96	0.25	<b>72.26</b>	72.76	0.50
GGTTCGCGAGCG	<u>90</u>	<b>62.15</b>	61.14	-1.01	<b>65.75</b>	64.22	-1.53